Fewer Pigmented Locus Coeruleus Neurons in Suicide Victims: Preliminary Results

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Studies of the noradrenergic system in suicide victims have found evidence of alterations in cortical β- and α-adrenergic receptor binding. Since these receptor changes may be secondary to altered noradrenergic input, we sought to determine whether the pigmented neurons of the locus coeruleus (LC), which provide the noradrenergic innervation to the cerebral cortex, are altered in suicide victims.

We studied 11 controls without known psychiatric or neurologic disorders and six suicide victims. LC neuron number, LC volume, and neuron density were determined by computer-assisted mapping.

The suicide group had 23% fewer LC neurons and a 38% lower density of LC neurons than controls. The reduction in neuron number was localized to the rostral two thirds of the LC. Neither the LC length nor the LC volume in suicide victims differed from controls.

Altered brain noradrenergic neurotransmission in suicide victims may be due to fewer noradrenergic neurons in the LC. Further studies are needed to determine whether this noradrenergic neuron loss is associated with an underlying major depression or specifically with suicidal behavior.

Key Words: Noradrenaline, human, brain, 3-D reconstruction, suicide

Introduction

A growing body of evidence suggests that suicidal behavior is associated with altered noradrenergic neurotransmission and reduced serotonergic activity. Increased binding to β-adrenergic receptors in the cerebral cortex in suicide victims compared to controls has been reported by some investigators (Mann et al 1986; Arango et al 1990; Biegon and Israeli 1988), but not by others (De Paermentier et al 1990; Stockmeier and Meltzer 1991; Little et al 1993). Some studies also report increased α_1-adrenergic and/or α_2-adrenergic receptor binding in suicide victims in cerebral cortex (Arango et al 1993a; Meana and García-Sevilla 1987). Too few studies of brain stem norepinephrine concentrations have been carried out to draw firm conclusions (Bourne et al 1968; Moses and Robins 1975). Some (Ågren, 1980, 1982), though not all (Roy et al 1985, 1989; Brown et al 1982; Pickar et al 1986; Secunda et al 1986; Träskman et al 1981), studies have found reduced concentrations of the principal norepinephrine metabolite 3-methoxy, 4-hydroxyphenylglycol (MHPG) in the cerebral spinal fluid (CSF) of suicide attempters. Excretion of

MHPG is reduced in the urine of suicide attempters (Ågren, 1980, 1982). Taken together, these studies suggest a relationship between reduced noradrenergic neurotransmission and suicidal behavior.

Noradrenergic innervation of·the mammalian cerebral cortex is derived nearly exclusively from pigmented neurons of the locus coeruleus (LC) (Dahlström and Fuxe 1964; Levitt and Moore 1978; Freedman et al 1975; Jones and Moore 1977; Porrino and Goldman-Rakic 1982). The colocalization of neuromelanin pigment and the noradrenaline biosynthetic enzymes tyrosine hydroxylase and dopamine-β-hydroxylase in immunocytochemical studies suggests that the pigmented neurons of the LC are noradrenergic in phenotype (Iversen et al 1983; German et al 1992). In addition to the cerebral cortex, these neurons provide widespread innervation throughout the neuraxis including limbic system. Noradrenergic neurons may play a role in mood, memory, and cognition; this is suggested by findings of fewer LC neurons in Alzheimer's disease (German et al 1992; Marcyniuk et al 1986; Chan-Palay and Asan, 1989a; Iversen et al 1983; Bondareff et al 1982), Parkinson's disease (German et al 1992; Chan-Palay and Asan 1989a; Gaspar and Gray 1984), depression (Chan-Palay and Asan 1989b), and alcoholism (Arango et al 1993b, 1994). The studies of the LC in depression and alcoholism are of particular relevance to suicide, given the high incidence of major depression and alcoholism in suicide victims (Murphy et al 1992). Given the LC's fusiform distribution of neuron density and topographical organization, a systematic rostrocaudal axis sampling of the nucleus is critical. In the present study we used such an approach to determine whether the alterations in brain noradrenergic function in suicide victims are associated with changes in the number of noradrenergic LC neurons.

Subjects and Methods

All procedures for collection of brain tissue were approved by the Institutional Review Board for Human Use Considerations of the University of Pittsburgh.

Tissue Collection

Brainstems ($n = 17$) were collected at autopsy from the coroner's office. All subjects were free of gross neuropathology and had negative toxicology screens in blood, urine, and bile for psychoactive or neurotoxic drugs.

Control subjects died of accidental death ($n = 6$), homicide ($n = 1$), or natural causes ($n = 4$) and had no evidence of drug abuse, neuropathology, or psychopathology based on toxicology, autopsy findings, and data obtained by the coroner's staff from next of kin and other sources (Table 1). Control cases ($n = 11$) ranged in age

Table 1. Characteristics of Subjects

No.	Sex	Age (y)	PMI	Race	BW	Cause of death	Total neurons[a]
Controls							
1	M	17	5.5	B	1,500	Firearm	45,589
2	M	19	14	B	1,490	MVA	41,952
3	M	22	24	W	1,600	Fall	47,495
4	M	25	12	W	1,330	MVA	43,394
5	M	29	20	W	1,600	Cardiac	44,575
6	M	42	15	W	1,500	Cardiac	41,576
7	M	43	18	W	1,480	MVA	39,255
8	M	43	17	W	1,230	Cardiac	47,402
9	M	54	9	W	1,460	MVA	39,516
10	F	65	21	W	1,150	MVA	39,367
11	M	69	22	W	1,415	Cardiac	48,072
Suicides							
12	M	23	16	B	1,450	Hanging	42,910
13	F	33	3	W	—	Hanging	34,697
14	F	43	12	W	1,480	Overdose	36,112
15	F	61	11	W	1,120	Hanging	34,522
16	F	75	15.5	W	1,020	Other	28,855
17	M	84	20	W	1,400	GSW	23,604

PMI = postmortem interval (time from death to fixation in hours); B = black; W = white; BW = brain weight (in grams); MVA = motor vehicle accident; GSW = gun shot wound.
[a]Total number of pigmented neurons in the left and right LC.

from 17–69 years (39 ± 5 y) and had a male:female ratio of 10:1. A determination of suicide was made by the county coroner and verified by the investigators based on the coroner's records. The suicide group ($n = 6$) did not differ in age range from controls (23–84 y; 53 ± 10 y; $F_{1,15} = 1.90, p = 0.19$), and the male:female ratio was 2:4 (Table 1). The postmortem interval (PMI) did not differ ($F_{1,15} = 1.18, p = 0.28$) between controls ($16 \pm 2$ h) and suicides (13 ± 2 h).

Upon removal of the brain from the cranium, the cerebellum was removed and the brainstem separated with a transverse cut at the anterior border of the superior colliculi. The brainstem was then fixed in formalin (10%) for 2 weeks. Following initial fixation in formalin, the brainstem was manually sectioned into 2–3-cm blocks to allow for introduction of fiducial markers and tissue block thickness limitations of the sliding microtome. Fiducial markers were placed to assist in section alignment for subsequent three-dimensional computerized reconstruction and consisted of five #000 stainless steel insect pins impaled through the blocks in the longitudinal axis. Tissue blocks were then infiltrated in increasing concentrations (10%–30%) of cryoprotectant sucrose, usually 5–7 days. Blocks were then sectioned on a sliding microtome (Microm Model HM400, Heidelberg, Germany) with sections (50 μm) collected every 100 μm. The sections were mounted on glass slides and stained with cresyl violet.

Morphometry

The methods used for morphometry have been described in detail elsewhere (Konigsmark 1970) and will only be summarized. Neuron counting was performed using a computerized camera lucida system (Neurolucida, Micro-Brightfield, Inc., Colchester, VT) attached to a microscope (Leitz Aristoplan) equipped with a motorized stage (Ludl). The protocol for estimation of neuronal population with correction for split cell error was adapted from Konigsmark (1970).

Computerized mapping of neuron distribution and three-dimensional reconstruction was performed using commercially available computer software (NeuRotate, MicroBrightfield, Inc.). To minimize counting error, sections were counted every 500 μm throughout the entire rostrocaudal extent of the nucleus. Within a section, neuromelanin-containing neurons with nucleoli were identified and marked with a pointing device interfaced with the microscope stage controller and a computer to establish the x, y, and z coordinates of each cell, the coordinates of the LC and section outlines. The distribution of LC neurons in controls and suicides was examined in two ways. First, the number of pigmented LC neurons was plotted as a function of the distance from the trochlear decussation. Second, because the rostrocaudal extent of LC varied between brains (range = 11.5-19.5 mm), the length of the LC was normalized according to the method of German et al (1992) such that the most caudal extent of the nucleus was considered 0% and the most rostral extent was 100%. The number of left and right pigmented LC neurons and the area of the left and right LC were extracted from the data file.

Data Analysis

Data are presented as mean ± SEM. Comparisons of the left and right LC neuron number, volume, length, and neuron density were made using a two-tailed paired t test. Group contrasts were based on the total number of neurons and the mean volume, length, and neuronal density, using an analysis of variance (ANOVA). Comparisons of the distribution of neuron density between groups were done by an ANOVA and post hoc t tests.

Results

The normal distribution and morphology of pigmented neurons in the human locus coeruleus (LC) is described in detail elsewhere (German et al 1988: Chan-Palay and Asan 1989b) and will therefore only be summarized here. Pigmented LC neurons appear in the dorsomedial pons at the junction between the pons and the cerebral peduncles. The neurons are located bilaterally and symmetrically in

the ventrolateral quadrants of the central gray. Proceeding in a caudal direction, the neurons' relative location moves laterally and ends approximately at the most rostral appearance of the motor subdivision of the trigeminal nucleus. The neurons of the LC proper are not evenly distributed throughout the rostrocaudal extent of the nucleus, which has a compact and tubular shape. The density of pigmented neurons reaches its maximum 3-5 mm caudal to the decussation of the trochlear nerves (Figures 1 and 2, upper).

In controls, the number and distribution of neurons did not differ in the left compared to the right LC ($p > 0.05$, paired t test; Table 2); however, in the suicide group, there were significantly fewer pigmented neurons in the left than the right LC ($p = 0.01$, paired t test; Table 2). This asymmetry was small (2%-13%) but was observed in every suicide case. Neither the LC volume (control: $p = 0.78$; suicide: $p = 0.10$, paired t test) nor the density of neurons (control: $p = 0.56$; suicide: $p = 0.94$, paired t test) were asymmetric in either group (Table 2). In controls, the LC was 16.1 ± 0.7 mm in length and had a total volume of 37 ± 3 mm^3. The estimated total number of LC neurons was 43,472 ± 1,021 and the average density of neurons was 1,227 ± 85 cells/mm^3.

In the suicides, the total number of LC neurons (33,450 ± 2,692) was significantly less than that of controls ($F_{1,15} = 17.62$, $p = 0.0008$). Five of the six suicides had fewer pigmented LC neurons than the control with the fewest cells (Figure 3). Similarly, the average density of neurons (794 ± 103 cells/mm^3; $F_{1,15} = 8.89$, $p = 0.0093$) was significantly lower in suicides than controls (Table 2).

To determine whether the reduction in the number of pigmented neurons was anatomically restricted or widespread, the number of neurons was examined every 500 μm throughout the length of the LC (Figure 2, upper). The difference in neuron number between the suicides and the controls was apparent both in the left ($F_{1,15} = 18.73$, $p = 0.0006$) and the right ($F_{1,15} = 15.64$, $p = 0.0013$) LC. Compared to controls, a statistically significant reduction in numbers of pigmented neurons appeared to be through the middle two thirds of the LC of suicide victims ($p < 0.05$, ANOVA, t tests, Figure 2, upper).

To address the possibility that the difference in the distribution of neurons between controls and suicides was due to misalignment of the LC with respect to the internal brainstem reference landmark (i.e., the trochlear decussation) or to differences in brainstem size, we reexamined the neuronal distribution following normalization of the LC length between groups (Figure 2, lower). Following this normalization procedure (German et al 1992), a similar reduction in suicide victims was observed but was now found clearly to be localized through the rostral two
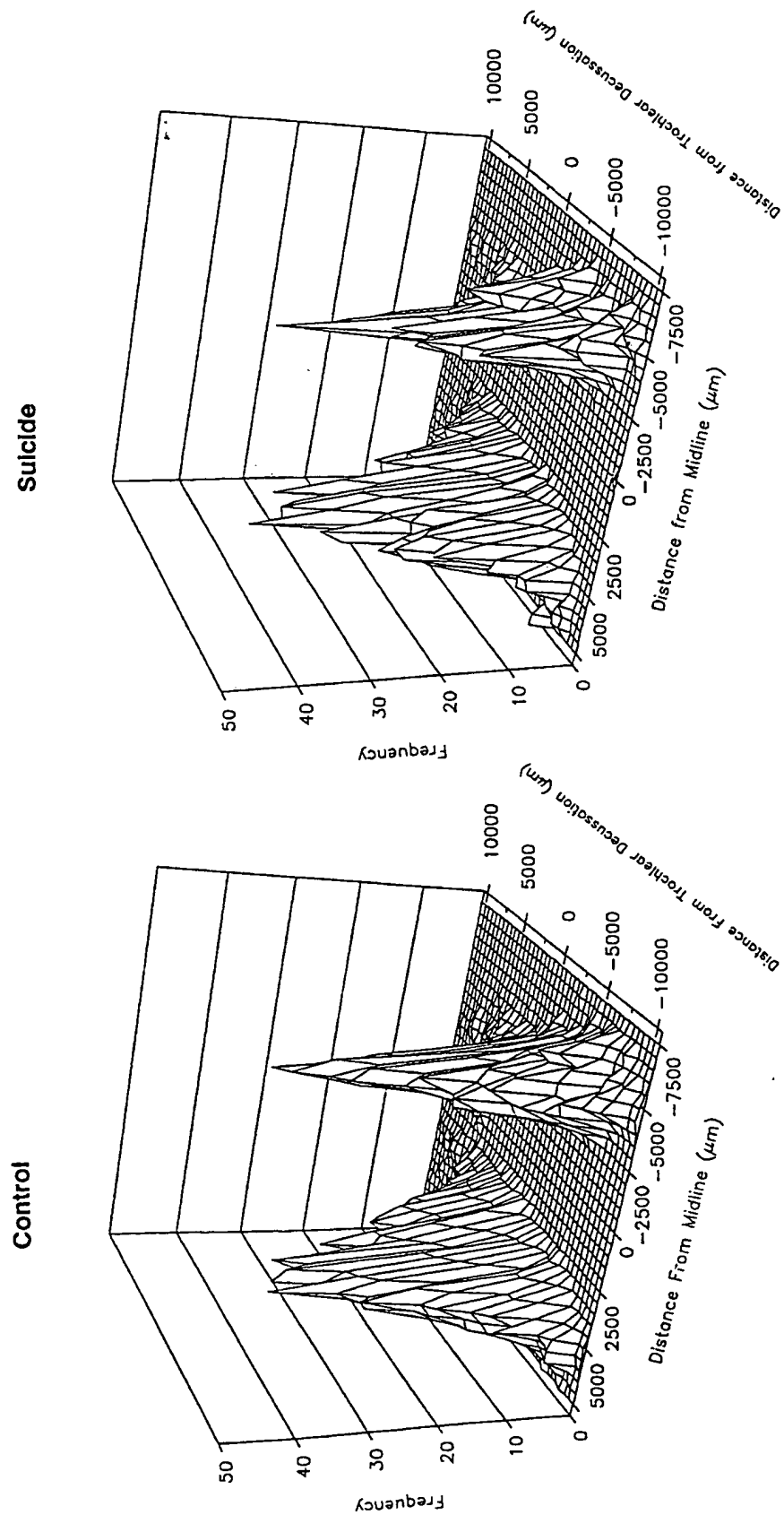
Suicide

Control

Figure 1. Distribution of pigmented LC neurons in the mediolateral and anteroposterior plane of controls (*left*, *n* = 11) and suicide victims (*right*, *n* = 6). Note that there are fewer LC neurons in the suicide victims throughout the anteroposterior extent of the LC, but not in the mediolateral plane.
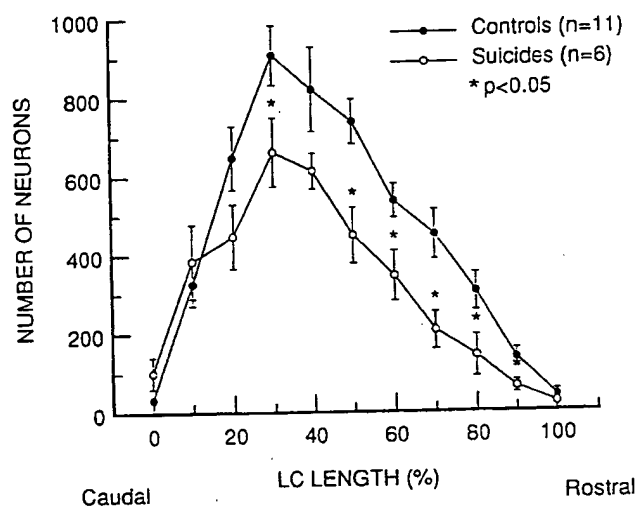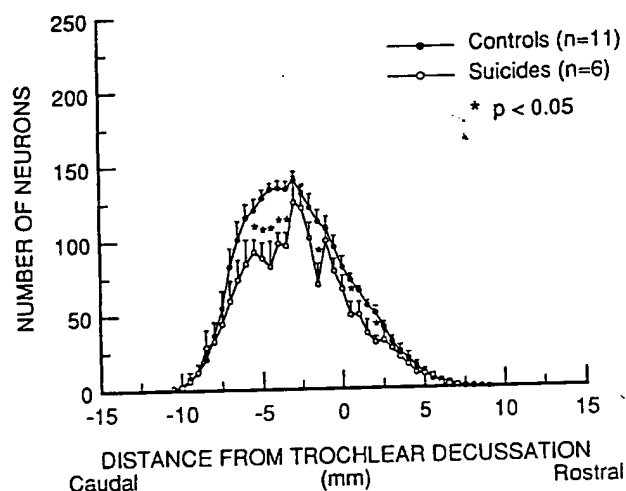
Figure 2. Distribution of the number of pigmented neurons in the locus coeruleus (LC) in controls and suicides plotted with respect to either an internal landmark (upper) or normalized as a function of the length of the LC (lower). Note that (1) the density of pigmented neurons was greatest in the caudal half of the LC in both groups, and (2) the reduction in neuron number in suicides was distributed throughout the middle two thirds of the LC.

thirds of the LC (Figure 2, lower). Neither the LC length (15.9 ± 0.8 mm; $F_{1,15} = 0.03$, $p = 0.86$) nor the total LC volume (46 ± 7 mm^3; $F_{1,15} = 2.05$, $p = 0.17$) was different from controls.

As has previously reported in control subjects (Vijayas-hankar and Brody 1979; German et al 1988), the total number of neurons in suicides was highly correlated with age ($r = 0.92$, $p = 0.009$; Figure 3); however, the slopes of the relationships between age and total number of neurons was different between controls and suicides ($p = 0.03$), and thus the reduction in neuron number in the suicides compared to controls appeared to be greater with increasing age (Figure 3). LC neuron number did not correlate with PMI (control: $r = 0.269$, $p = 0.42$; suicide: $r = -0.361$, $p = 0.48$) or brain weight (control: $r = 0.159$, $p = 0.64$; suicide: $r = 0.352$, $p = 0.59$) in either group.

Discussion

Using quantitative computer-assisted morphometry, the present study demonstrates fewer pigmented neurons in the locus coeruleus of suicide victims compared to controls. The difference in LC neuron number was most pronounced in the rostral two thirds of the LC. Neurons in the rostral LC are topographically organized and project to the ipsilateral cerebral cortex, while neurons in the caudal LC project predominantly to hindbrain structures such as the cerebellum, brainstem, and spinal cord (Dahlström and Fuxe 1964; Levitt and Moore 1978; Freedman et al 1975; Jones and Moore 1977; Porrino and Goldman-Rakic 1982). Thus, the effect of this cell loss is likely to result in effects on the cerebral cortex, and unlikely to be detected in changes involving peripheral noradrenergic function emphasizing the importance of direct brain studies. The left–right difference in LC neuron number found in every suicide victim but not in the control group further highlights the selectivity of the changes in the LC and the specific topography of these changes on the noradrenergic terminal fields.

Table 2. Morphometry of the Locus Coeruleus (LC) in Controls and Suicides

	Controls ($n = 11$)		Suicides ($n = 6$)	
	Left LC	Right LC	Left LC	Right LC
Neuron number	21,677 ± 522	21,794 ± 529	16,297 ± 1,414[a,b]	17,152 ± 1,284[a]
Volume (mm^3)	18.6 ± 1.4	18.7 ± 1.4	22.4 ± 3.3	23.5 ± 3.3
Neuron density (cells/mm^3)	1,236 ± 96	1,218 ± 85	794 ± 112[a]	795 ± 103[a]

Values are mean ± SEM.
[a] $p < 0.05$ from control, t test.
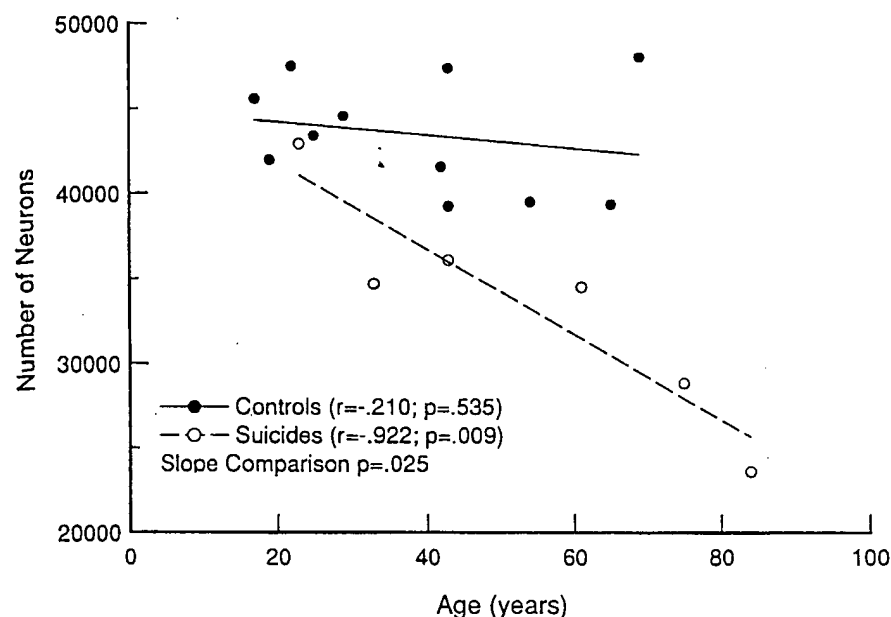[b] $p < 0.05$, left vs. right, paired t test.

Figure 3. Relationship between age and the total number of pigmented neurons in the locus coeruleus in controls and suicide victims. Note that there is a correlation between age and the total number of pigmented neurons in suicide victims but not in controls.

Studies of the neurobiology of suicide have suggested that alterations in the serotonergic and noradrenergic systems in brain may constitute a significant risk for suicidal behavior (Arango and Mann 1992; Mann and Arango 1992). With respect to the noradrenergic system, the findings of reduced NE metabolites in the CSF and urine (Ågren, 1980, 1982) and increased numbers of β-adrenergic receptors for NE (Mann et al 1986; Arango et al 1990; Biegon and Israeli 1988) in suicides suggest reduced noradrenergic function. The finding in this study of fewer noradrenergic neurons in suicides is consistent with, and may be responsible for, altered noradrenergic indices in suicides.

The noradrenergic neuron loss in suicide may be related to the predisposition for acting on suicidal thoughts or due to the presence of a psychiatric disorder such as major depression. Reduced noradrenergic function has long been thought to contribute to depressive illness (e.g., Homykiewicz 1974). A recent pilot study reported a single case of an 86-year-old depressed patient with fewer neurons in the LC postmortem (Chan-Palay and Asan 1989b). Given that the incidence of depression in suicide victims is approximately 60% (e.g., Barraclough et al 1974; Robins et al 1979; Dorpat and Ripley 1960), it is possible that perhaps four of the six suicides in this study suffered from a depression disorder. A single case report (Chan-Palay and Asan 1989b) is too preliminary to permit any definite conclusion as to whether our findings are due to the presence of an associated major depression. Further studies are needed on brain samples from subjects with and without major depression.

Two other studies have examined the number of LC neurons in suicide victims (Biegon and Fieldust 1992; Ordway et al 1994). Biegon and Fieldust (1992) reported reduced intensity of tyrosine hydroxylase (TH) immunoreactivity and no differences in the number of TH-positive LC neurons in suicide victims. Ordway et al (1994) found an increase in the concentration of TH in the LC and no difference in the number of pigmented LC neurons of suicide victims, and these authors concluded that the concentration of TH per neuron was elevated in suicide; however, the neuron counting in both studies (Biegon and Fieldust 1992; Ordway et al 1994) was performed only on two to three sections of the LC per case; since we found that the reduced number of LC pigmented neurons is anatomically restricted, it is possible that the sections studied were outside the region of the LC where differences are detectable in suicide victims. The finding of reduced amounts of TH immunoreactivity (Biegon and Fieldust 1992) would support the hypothesis that there is reduced noradrenergic function in the brain of suicide victims at the time of suicide. Alternatively, the observation of elevated TH per neuron by Ordway et al (1994), taken in conjunction with our finding of an anatomically discrete loss of LC neurons in suicide, suggests that the remaining LC neurons may upregulate enzyme synthesis. Further studies are required to distinguish between these possibilities.

In the present study we found fewer (6%) pigmented LC neurons in the left LC than the right LC in every suicide case. No such asymmetry was found in the controls. This asymmetry has not been previously reported. There is

evidence suggesting a relationship between the development of depression and the presence of lesions involving the left frontal cortex (Sackeim et al 1982; Robinson et al 1984; Starkstein et al 1988) and/or left subcortical regions (Starkstein et al 1987). Furthermore, excitatory effects due to an epileptic focus in the right hemisphere are associated with pathological crying (Sackeim et al 1982). Robinson et al (1984) have hypothesized that the depression following asymmetrical frontal lobe lesions might be the result of damage to brainstem catecholamine pathways innervating the cerebral cortex which produce a reduction in catecholamines in cortical regions, resulting in negative affect. Our finding of an asymmetric, greater left-sided decrease in LC neurons in suicide victims suggests that suicide victims might have relatively more left frontal lobe dysfunction related to a disproportionate reduction in noradrenergic innervation to the left hemisphere, which may in turn contribute to a depressive disorder. Studies of α- and β-adrenergic receptor binding and norepinephrine in cerebral cortex have not addressed the question of left–right differences.

One of the six suicide cases in our study had a "normal" number of LC neurons. A possible explanation for this case is that a noradrenergic deficiency represents only one of the several major risk factors (e.g., Mann and Arango 1992).

The difference in neuron number between the suicide and control group cannot be accounted for by differences in age or postmortem delay, since these variables did not differ between groups. An age-related reduction in the number of LC neurons has been reported (Vijayashankar and Brody 1979; German et al 1988). We did not observe an age-related loss of LC neurons in controls since previous reports (Vijayashankar and Brody, 1979; German et al 1988) have found that this loss of neurons does not occur until after the seventh decade of life, and our oldest control case was 69 years. Our finding of an age-related reduction in the number of LC neurons in the suicide group does not appear to be explained by the two suicide cases older than 70 years because, as Figure 3 indicates, the age-related neuron loss is evident in cases under the

age of 70 years. More probably, the finding suggests an interaction between age, LC neuron number, and suicidality whereby, relative to younger suicides and controls, the older suicides have a greater reduction in the number of noradrenergic neurons compared to age-matched controls.

The difference between groups is not likely due to sex differences in the group compositions, since differences in the number of LC neurons in males and females were not observed by ourselves or others (German et al 1988, 1992). The results cannot be due to the presence of recently ingested drugs or neurotoxic agents, because both groups were determined to be drug-free by history and by toxicological screens of blood, urine, bile, or intraocular fluid; however, long-term effects of drugs cannot be ruled out. The possibility that LC neurons in suicides lost pigment but did not undergo neuron death is unlikely, since pigment loss without neuron loss has not been observed in other diseases associated with LC neuron loss, such as Alzheimer's disease, Parkinson's disease, and Down syndrome (German et al 1992; Iversen et al 1983).

In summary, the present study has demonstrated a reduction in the total number and density of neuromelanin-containing neurons of the LC in suicides compared to controls. These findings suggest that suicides may have reduced noradrenergic function in the cerebral cortex, particularly on the left side, and other brain regions due to a loss of noradrenergic neurons in the LC. Studies are in progress comparing suicides with and without major depression to determine whether major depression is associated with this noradrenergic cell loss.

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